

RISK FACTORS AND REFERENCE VALUES OF ANTIBIOTIC RESIDUES IN IMPORTED RED MEAT AND THEIR IMPACTS ON GENOTOXICITY AND DNA DAMAGE IN HUMAN LYMPHOCYTES

May R. Hawas¹
Researcher

Firas R. Al-Samarai²
Prof.

¹Health Cont. Devi .Min. of Heal. Baghdad, Iraq.Email:

²Dept. of Veter.Publ.Heal.,Coll.Vet. Med.University of Baghdad, Iraq.

may.rasheed1104e@covm.uobaghdad

firasrashad@vet.uobaghdad.edu.iq

ABSTRACT

Antibiotic residues in food are accelerate the development of antibiotic resistance in bacteria, transfer of antibiotic-resistant bacteria to humans, cause allergies (penicillin), and cancers. This study was designed to investigate the risk factors of some antibiotic residue in imported red meat at Baghdad markets, their reference values, and their impact on genotoxicity and DNA damage in human cells. A total of 144 meat samples were collected from January to July 2022. The results showed that the overall contamination percentage was 35.4%. Buffalo meat showed the lowest contamination percentage 15% whereas the minced beef showed the highest (94.5%) (OR=402.33). Tetracycline residues detected in 42.8% of samples with significant risk (P=0.0001) (OR=33.37; 95% CI=7.74-143.94). The mean of tetracycline was 83.80 ppb with lower and upper limits of 56.7, 110.9 ppb, respectively. Blood samples of 18 healthy males were used to isolate lymphocytes to detect the genotoxicity and DNA damage of antibiotic residues. Results showed significant effects of antibiotic residues on genotoxicity and human DNA damage. These results also showed that although the antibiotics residues concentrations are below the allowed maximum residues limit, this does not reduce the hazards of these residues on public health resulting from their accumulative increase in consumers' tissues.

Keywords: antibiotic residues, reference values, genotoxicity, red meat, Iraq, food safety

هواس والسامرائي

مجلة العلوم الزراعية العراقية- 2025 56: (2) 905-915

عوامل الخطر والقيم المرجعية لبقايا المضادات الحيوية في اللحوم الحمراء المستوردة وتأثيراتها على السمية الجينية وتلف الحمض النووي في خلايا الإنسان

فراس رشاد السامرائي

مي رشيد هواس

أستاذ

باحث

كلية الطب البيطري/ جامعة بغداد

قسم الرقابة الصحية/ وزارة الصحة

المستخلص

تساهم بقايا المضادات الحيوية في الأغذية في تسريع تطور مقاومة المضادات الحيوية في البكتيريا، ونقل البكتيريا المقاومة للمضادات الحيوية إلى البشر، وتسبب الحساسية (البنسلين)، والسرطانات. صُممت هذه الدراسة للتحري عن عوامل الخطر لبعض بقايا المضادات الحيوية في اللحوم الحمراء المستوردة في أسواق بغداد، وقيمتها المرجعية، وتأثيراتها على السمية الجينية وتلف الحمض النووي في الخلايا البشرية. تم جمع 144 عينة لحوم من كانون الثاني إلى تموز 2022. أظهرت النتائج أن نسبة التلوث الكلية كانت 35.4%. أظهر لحم الجاموس أقل نسبة تلوث 15% بينما أظهر لحم البقر المفروم أعلى نسبة (94.5%) (OR=402.33). تم الكشف عن بقايا مضاد التتراسيكلين في 42.8% من العينات مع خطر معنوي (P=0.0001) (OR=33.37; 95% CI=7.74-143.94). وبلغ متوسط تركيز التتراسيكلين 83.80 جزء في المليار مع حد أدنى وأعلى 56.7, 110.9 جزء في المليار على التوالي. وتم استخدام عينات دم 18 ذكر سليم لعزل الخلايا الليمفاوية للكشف عن السمية الجينية وتلف الحمض النووي لبقايا المضادات الحيوية. وأظهرت النتائج تأثيرات كبيرة لبقايا المضادات الحيوية على السمية الجينية وتلف الحمض النووي البشري. وأظهرت هذه النتائج أيضاً أنه على الرغم من أن تراكيزات بقايا المضادات الحيوية أقل من الحد الأقصى المسموح به للبقايا، إلا أن هذا لا يقلل من مخاطر هذه البقايا على الصحة العامة الناتجة عن زيادتها التراكمية في أنسجة المستهلكين

الكلمات المفتاحية: بقايا المضادات الحيوية، القيم المرجعية، السمية الجينية، اللحوم الحمراء، العراق، سلامة الغذاء

Received:7/10/2024, Accepted:8/1/2025

INTRODUCTION

Antimicrobials are widely used to control, prevent, and treat infection, and to enhance animal growth and feed efficiency (33). As a result of the extensive use, the antibiotics residues are often found in animal products, such as meat, milk, and eggs that eventually find humans to be the ultimate consumers of these antibiotic residues (39, 38). Red meat and meat products are considered good foods with high nutritional value because they contain vitamins, proteins, fats, and minerals. The available local red meat is not enough to meet consumer demand. (1, 3, 6, 28). Consuming foods including red meat that contaminated with antibiotic residues could increase bacterial resistance to antibiotics, posing a significant public health risk (9, 43, 24). These antibiotic-resistant bacteria were transferred to the human and can cause severe and often fatal infectious diseases (16). Ramatla *et al.*, (44) detected different concentrations of antibiotic residues using HPLC that were in the ranges of 20.7–82.1, 41.8–320.8, 65.2–952.2, and 32.8–95.6 µg/kg for sulphanilamide, tetracycline, streptomycin, and ciprofloxacin, respectively. In Iraq, Ahmed *et al.*, (7) found the antibiotic residues in blood of sheep (0.413 ppb), and heifers (0.358 ppb). Antibiotic residues as genotoxic agents could cause DNA damage in cells, leading to mutations and cancer (14). Assessing the genotoxic potential of food contaminants including meat is crucial for understanding their impact and risk on human health (19, 13, 36). Micronucleus (MN) tests are used to detect chromosome fragments in cells to demonstrate potential genetic damage (45). Chromosomal Aberration tests identify structural changes in chromosomes, revealing mutagenic effects of antibiotic residues in foodstuffs (29). DNA damage can be detected by comet assay through gel electrophoresis (18). These tests were used to detect the gene toxic effects and DNA damage induced by antibiotic residues in red meat in the Baghdad markets. This research was conducted to cover the great gap in Iraqi studies that investigate the effects of antibiotic residues on genotoxicity and DNA

damage in human lymphocytes.

MATERIALS AND METHODS

In this study, a total of 144 imported red meat samples were collected from various markets across Baghdad (Al-Karkh and Al-Rusafa districts). The sample collection spanned from January to July 2022 and included beef, cows, buffalos, veal, and minced beef.

Preparation of meat samples

The homogenized sample (10g) was transferred to a shaking bottle, a solution of a cetronitrile containing 1% formic acid (50 mL) was added, and the resulting mixture was shaken for 30 min. After this time, anhydrous magnesium sulphate (4 g) and sodium chloride (1 g, to increase the ionic strength and distribution efficiency) were added, and the mixture was shaken for 10 min prior to centrifugation for 10 min at 4000 rpm. The supernatant (25 mL) was then added to acetone containing 2% diethylene glycol (0.2 mL) and the solvent was evaporated to dryness. The resulting extract was re dissolved in a mixture of acetone/hexane (2:8, v/v, 4 ml), which was subsequently loaded onto an SPE-florisil cartridge that was activated with hexane (5 ml) and acetone/hexane (2:8, v/v, 5 ml) for sample purification.

Liquid Chromatography - Mass Spectrometry (LC/MS): The ultra-high-performance liquid chromatographic system by Liquid chromatography- tandem mass spectrometry LC-MS/MS AGILENT 1290 USA coupled with the QTRAP®6500+ MS system from AB Sciex (Framingham, MA, USA) was used as standards method for analysis of contamination with antibiotic residues. Blood samples collected from 18 healthy individuals were used to isolate lymphocytes for conducting genotoxicity test (49)

Genotoxicity effects and DNA damage analysis

1- The Micronucleus (MN) test was applied according to Fenech (22), Al-Sudany (11) and Ad'hiah *et al.*, (5) to estimate the MN formation. Briefly, after cell preparation and addition of cytochalastin B, they were fixed and smeared on clean slides followed by staining with Giemsa. The slides were

examined under oil immersion lens (100X), and inspected for the formation of micronucleus. The micronucleus index was scored using 1000 cells by using the following equation:

$$\text{Micronucleus index (micronucleus/cell)} = \left(\frac{\text{Number of Micronuclei}}{\text{Total Count of Cells}} \right) \times 100$$

2- The Mitotic Index (MI) and chromosomal Aberration (CA) were estimated according to Shubber (49). In brief, the estimation of the MI and CA in human blood, cells were prepared, PHA stimulated and olchirin addition and then harvested to be fixed and stained by Giemsa, then examined microscopically. The percentage rate for only the divided cells was then determined using the formula below:

Mitotic index=

$$\left(\frac{\text{Number of the divided cells}}{\text{Total number of the cells}} \right) \times 100$$

3- DNA damage can be detected by comet assays after mounting cells, microscope slides in a thin layer of agarose gel, the cells were lysed to eliminate all cellular proteins. The DNA was unwound, electrophoresed, and then fluorescently dyed after that relaxed chromatin or fragmented DNA fragments (damaged DNA) migrate away from the nucleus during electrophoresis. The amount of DNA released from the comet's head directly relates to the amount of DNA damage.(10).

Statistical analysis

Data were analyzed using SAS program (version 9.1) (46). Independent t test was used to assess the significant difference between two means and Chi-square test was used for proportions. If any expected frequency is less than 5 in more than 20% of cells, the Chi-square tests is inappropriate, therefore, it is usually considered acceptable

if Yates' correction is employed. Odds ratio were estimated using MedCalc program (35). Reference values were estimated by using reference value advisor (25). $P < 0.05$ is considered significant.

RESULTS AND DISCUSSION

In the current study, the Liquid Chromatography-Mass Spectrometry (LC/MS) was utilized to quantitative and qualitative detect the presence of antibiotics in various meat samples. This qualitative analysis provided valuable insights into the nature and types of antibiotic residues that present in meat products which may assist in the assessment of food safety and compliance with regulatory standards. Table(1) provides a comprehensive overview of the results to determine the contamination of different meat types with antibiotics residue. The antibiotics residue contamination contributed to 35.4% of all meat types. The minced meat had the highest percentage of antibiotics residue 94.5% (OR=402.33; 95% CI=39.4-4111.5) and the cow meat had 66.6% of contamination (OR=11.33; 95% CI=2.04-63.08) followed by veal meat of 24.4% (OR=136.8; 95% CI=28.3-653.5) and the lowest contaminated meat was detected in buffalo meat with a percentage of 15%. The high percentage of contamination of minced meat could be attributed to the processing practices of minced meat, which often involves mixing meat from multiple sources and handling and mixing processes (31). This ratio of contamination in cow meat was more than that observed in buffalo meat suggests that cows may be subjected to more intensive antibiotic treatments and difference in life span as well as rearing circumstance compared to other livestock (30, 37).

Table1. Percentages and health risk of contamination of meat with antibiotics residue in Baghdad meat market

Type of meat	Total No.	Antibiotics No.(%)	Yates Chi-Square	Yates P-value	Odds Ratio (95%CI)	P-value
Buffalo	20	3(15%)	36.01	<0.0001	Reference	
Cow	12	8(66.6%)			11.33(2.04-63.08)	0.005
Veal	94	23(24.4%)			136.8(28.3-653.5)	<0.0001
Minced	18	17(94.4)			402.33(39.4-4111.5)	<0.0001
Total	144	51(35.4%)				

Table (2) shows the contamination rates for various types of antibiotics residue. In

minced meat samples, it was noted that the highest contamination rate with Tetracycline residue (42.8%) with significant risk ($P=0.0001$) $OR=33.37$; 95% $CI=7.74-143.94$, followed by bi- combination contamination 28.6% ($OR=17.80$; 95% $CI=4.09-77.68$; $P<0.0001$), and tri- combination 19.6% ($OR=10.22$; 95% $CI=2.28-45.69$; $P=0.002$), then macrolides at a non- significant rate of

8.8% ($OR=3.31$; 95% $CI=0.67-16.38$; $P=0.14$) and the lowest was sulphonamides at 2.2%. The significant risk of abundant tetracycline residue in red meat could be explained by the over use of this antibiotic in disease prevention and treatments (47). Many studies reported that the tetracycline levels were above the maximum residual limit (8, 20, 12).

Table 2. Percentage and the risk factors associated with different types of antibiotics residue present in red meat contamination

Type of antibiotics	Contamination NO. (%)	Chi-square	P-value	Odds Ratio	P-value
Sulphonamides	2(2.2%)	48.50	<0.0001	Reference=1	-
Macrolides	8(8.8%)			3.31(0.67-16.38)	0.14NS
Tetracycline	39(42.8%)			33.37(7.74-143.94)	<0.0001
Bi-combination Tetracycline +Penicillin	26(28.6%)			17.80(4.09-77.68)	0.0001
Tetracycline +Sulphonamide					
Tetracycline +Macrolides					
Aminoglycoside+Macrolides					
Sulphonamide+Aminoglycoside					
Tri-combination Tetracycline+Penicillin+Macrolides Sulphonamide+Macrolides+ Aminoglycoside	18 (19.6%)			10.22(2.28-45.69)	0.002
Total	93 (64.6%)				

The reference values of the Tetracycline (sample number=63) and penicillin (sample number=24) were determined using reference value advisor. Results showed that Tetracycline had a lower limit of 56.7 ppb and an upper limit of 110.9 ppb with normal distribution (Figure 1), and LC/MS profile of Tetracycline was shown in Figure (2). The lower limit of penicillin was 10.62 ppb

and the upper limit was 11.48 ppb (Table 3) with also normal distribution (Figure 3) and LC/MS profile of penicillin was shown in Figure (4). In general, these values are considered below the acceptable maximum residue limit (MRL) of codex, which are 100 and 50 ppb for tetracycline and penicillin, respectively.

Table3. Reference values of tetracycline and Penicillin using reference value advisor

Type of antibiotic	No	Mean± STD ppb	Lower limit(LL)	Upper limit(UL)	90%LL	90%UL	Method (ppb)
Tetracycline	63	83.80±13.5	56.7	110.9	52.5-61.0	106.3-115.2	Codex
Penicillin	25	11.05±0.21	10.62	11.48	10.51-10.73	11.36-11.60	100 Codex 50

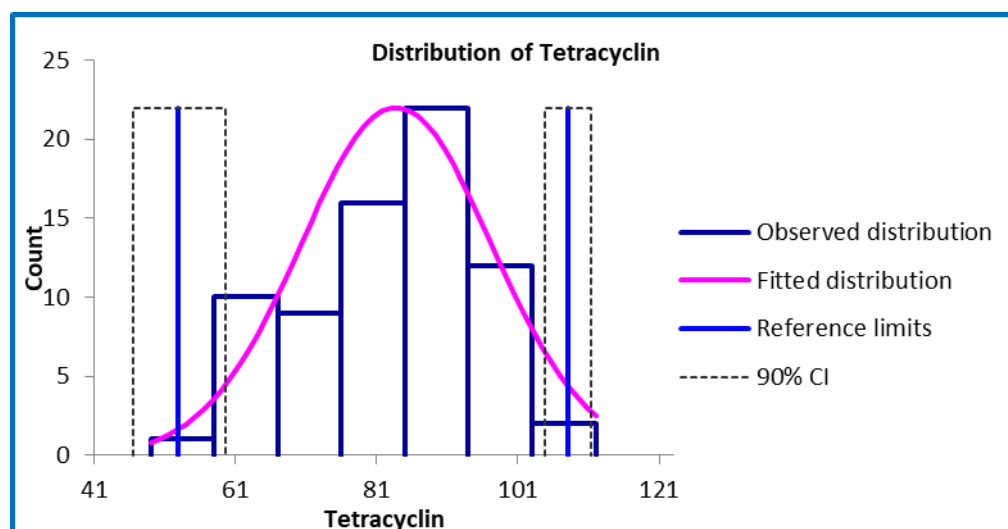


Figure1.The distribution of tetracycline (normal distribution)

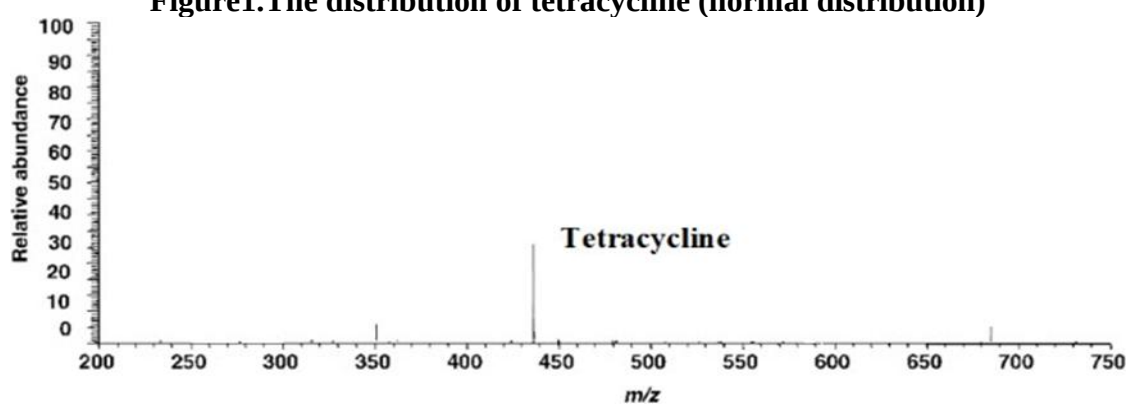


Figure2.LC–MS/analysis of Tetracycline residue in red meat.

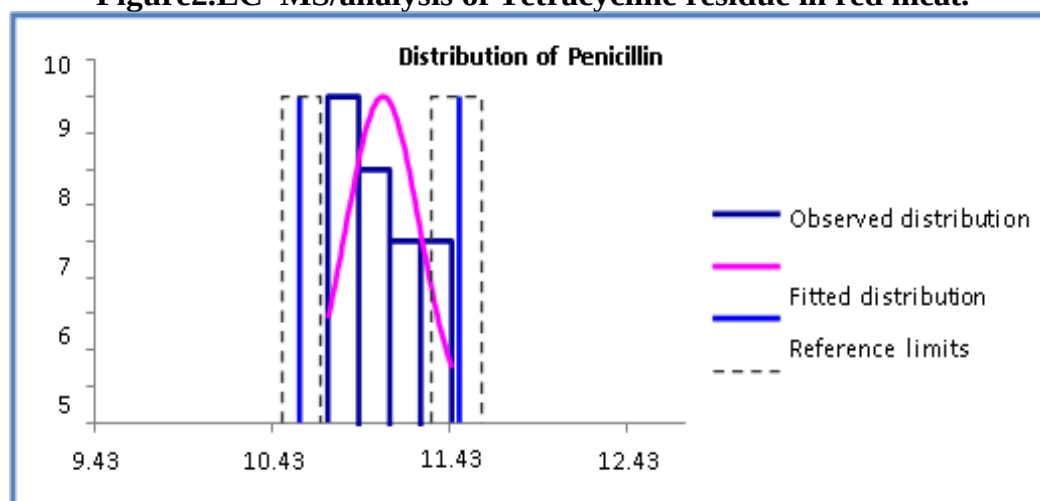


Figure3.The distribution of penicillin (normal distribution)

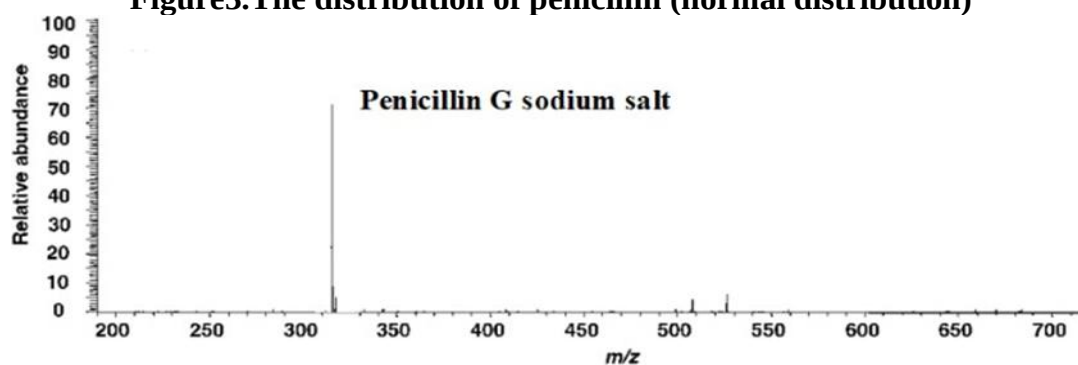


Figure4. LC–MS analysis of Penicillin residue in red meat

Overall, our study contributes to the ongoing discourse on food safety and agrees with the urgent call for comprehensive surveillance and strict enforcement of antibiotic use in live stock to ensure food safety and public health security. These efforts are crucial to mitigate the risks that are associated with antibiotics residue and to prevent the escalation of antibiotic resistance globally. In a study carried out by Er *et al.*, (20) in Turkey, the quinolone antibiotics residues were detected in 57.7% of beef meat in Ankara. Moreover, the misuse of antibiotics as a tool of promoting livestock production lead to deposition in red meat then cause a serious food safety concern due to their potential adverse health effects on consumers (12). Antibiotic residues can induce allergic reactions, promote the development and spread of antibiotic-resistant bacteria, and even cause severe health issues such as cancer, anaphylactic shock, reproductive disorders and chronic toxic effects on human health, such as liver or kidney damage (32, 26). The interaction between drug-drug could be another contributing factor that influencing the pharmacokinetics and clinical effects of drugs metabolism via either induction and/or inhibition of cytochrome P450 (CYP450) (34). It is interesting to know that antibiotics residue can remain in the animal's tissues and end up in the meat that is consumed by humans (44,42). In Iraq, a recent report by Wali and Deri (52) found that contamination rate of Tetracycline was 18.12% while Sulphonamide was 11.87% in beef meat, minced meat, and kebab. It has been found that 22.2% of examined sheep meats had antibiotics in Erbil, north of Iraq, which is considered a high.

Impact of Antibiotics Residue in meat on some Genotoxicity and human DNA damage: The genotoxic effects of antibiotics residue in red meat were examined using three different genotoxic

markers :the chromosomal aberrations (CA), micronuclei (MN), and micronucleus incidence (MI) (Table 4). Results revealed that the MN ratio had a significant increase in antibiotics-contaminated samples ($0.007 \pm 0.0005a$) indicating a high potential for genotoxic risk. Specifically, samples contaminated with antibiotics residue showed an increase in CA from 0.14 ± 0.02 of the free-contaminated samples up to 0.20 ± 0.02 of those contaminated with antibiotics residue. They also had an increase in the number of MN from 4.74 ± 0.29 to 8.19 ± 0.53 indicating significant changes in genotoxic markers and confirming a pronounced genotoxic health risk. The difference in MI between two groups was not significant. The presence of antibiotics residue in red meat and the associated genotoxic effects can have significant public health implications, such as mutagenesis and carcinogenesis (23), antibiotic resistance (17), reproductive and developmental toxicity (2, 27), or autoimmune disorders (4). In addition, antibiotic residues, such as tetracycline's and sulphonamides, can be mediated through DNA strand breaks and oxidative DNA damage in mammalian cells (51, 36). Furthermore, antibiotic residues can increase DNA damage markers, such as micronuclei formation and comet assay parameters, in various animal tissues (26, 48). Some antibiotics can also impair DNA repair mechanisms, allowing DNA damage to accumulate in cells (21). To alleviate the risks associated with antibiotics residue in red meat, strict regulations, monitoring programs, and proper withdrawal periods for treated animals are crucial. Additionally, promoting responsible antibiotic use in live stock production and exploring alternative strategies for disease prevention and control can help reduce the presence of antibiotics residue in the food supply (50).

Table 4. The effect of antibiotics residue on genotoxic effect on micronuclei (MN), chromosomal aberrations and MI in human cells

Status	No	CA	MI	No of cells Contain MN	No. of MN	Ratio MN/1000
Contaminated-free	26	0.14 ± 0.02	9.98 ± 0.05	$4.15 \pm 0.31b$	$4.74 \pm 0.29b$	$0.004 \pm 0.0003b$
Antibiotics	51	0.20 ± 0.02	9.92 ± 0.04	$5.68 \pm 0.29a$	$8.19 \pm 0.53a$	$0.007 \pm 0.0005a$

P-value	144	0.06	0.36	0.001	0.0001	0.01
---------	-----	------	------	-------	--------	------

The chromosomal aberrations which lead to genotoxicity were also observed in meat samples which was detected as gaps in chromosome and chromatid (Figure 3), representing breaks affecting the entire chromosome, but not completely separating any part.

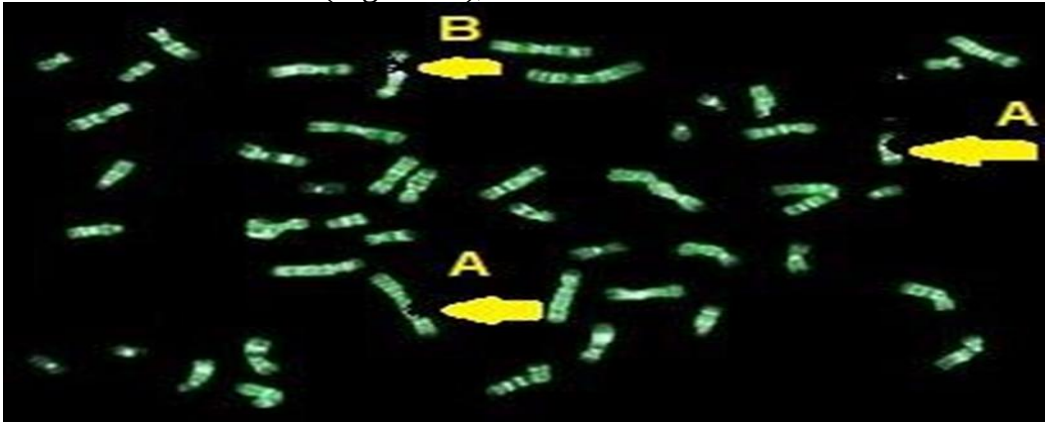


Figure 3. Chromosomal aberrations in red meat(100X).A.chromatid gaps (G'). B. chromosome gaps (G'') affecting whole chromosomes. Marked as "A"are chromatid gaps (G'), which are clear breaks within a single chromatid of a chromosome. Marked as "B" are chromosome gaps (G'').

The micronuclei results (Figure 4) showed that each cell has at least one micronucleus. The presence of this micronucleus indicates that the cell has undergone some form of chromosomal breakage or malfunction during cell division.

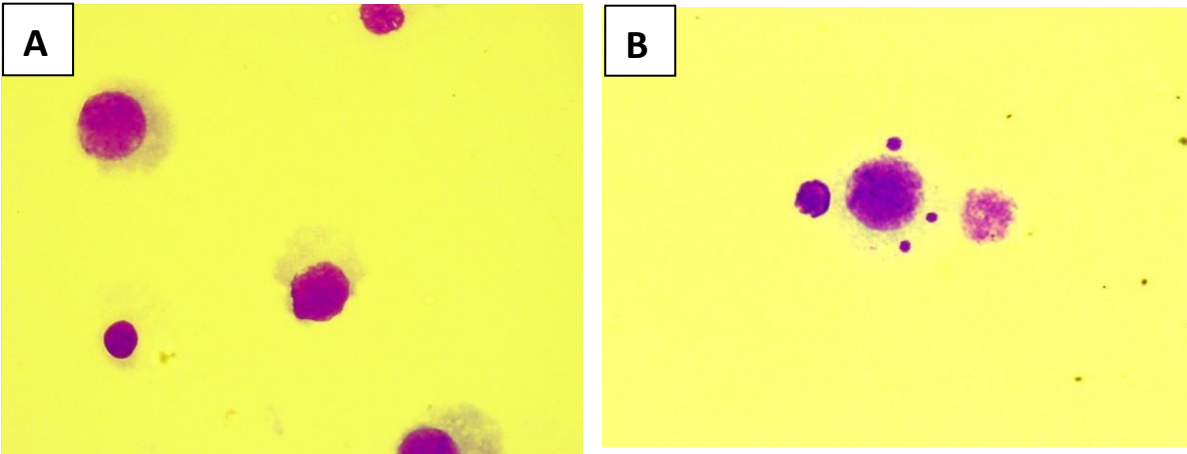


Figure 4. Micronuclei appearance in cells exposed to genotoxic agents (antibiotics). A. normal cells. B. cells with multiple micronuclei

Impact of Antibiotics Residue in red meat on DNA damage in human cells

Results of antibiotics residue contamination indicate a higher DNA damage in human lymphocytes;25.27±3.84% in the head and

27.56±3.25% in the tail (Table 5).These values are higher compared to 20.21±2.22 % and 16.24±2.65 % in free contaminated samples, respectively.

Table5.The effect of antibiotics on DNA damage in human by using comet assay

Status	Sample No	%DNA damage in head	%DNA damage in tail
Contamination. free	26	20.21±1.22b	16.24±2.65b
Antibiotics	51	25.27±1.24a	27.56±3.25a
P-value		0.01	0.02

Figure(5),shows the difference between normal and abnormal cells for DNA damage in head and tail, where normal cells are round and uniform in appearance with evenly distributed fluorescence suggesting undamaged cells (Figure 5A),whereas those

contaminated with antibiotics residue showed abnormality with irregularly shape and vary in size, some showing intense

fluorescence and others more diffuse fluorescence, suggesting DNA damage (Figure 5B).

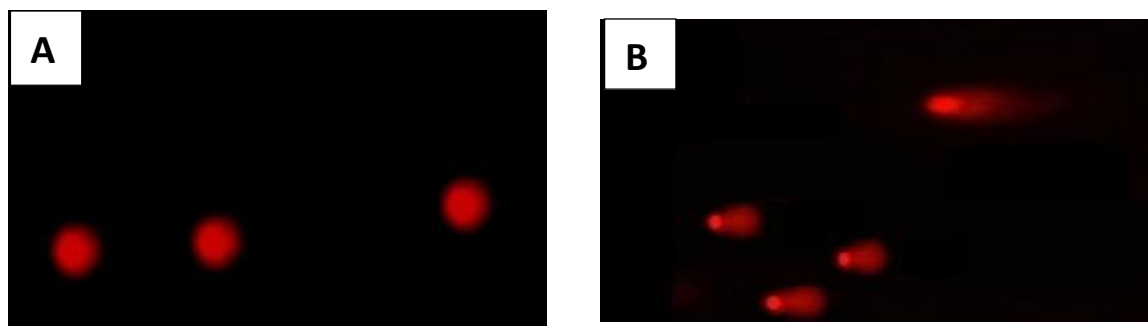


Figure 5.A. Control group showed no DNA damage in control group cells. B. Antibiotics residue contaminated meat samples with a significant migration of DNA from head to tail

The results concerning genotoxic effects and DNA damage agrees with several studies (41, 32, 15). The mechanism of DNA damage due to antibiotic residues in red meat could be caused by oxidative stress generating reactive oxygen species (ROS) which can directly damage DNA by causing strand breaks, base modifications, and DNA-protein cross-linking (44). Certain antibiotics or their metabolites can form DNA-reactive intermediates that covalently bind to DNA, causing adducts and mutations (40). Additionally, antibiotics like fluoroquinolones can inhibit topoisomerase enzymes, the crucial for DNA unwinding and replication leading to DNA double-strand breaks and chromosomal aberrations (53). Finally, some antibiotics can impair DNA repair mechanisms, allowing DNA damage to accumulate in cells (21). Therefore, the consistency of our results with these studies indicates the genotoxic potential of antibiotics residue in meat, aligning with broader concerns about antibiotic using in agriculture, meat production and its risk implications on human health. Although global studies are interested in studying the impact of antibiotics residue on public health, there has been a significant gap in local researches. Our study fills this critical void by providing an initial insights into how antibiotics residues in consumed meat causes genotoxic affect and DNA damage in humans. In addition, Tetracycline and bi-combination antibiotics residues were significantly present in food samples with 42.8% and 28.6%, respectively.

REFERENCES

1. Al-ghanimi, G. M. M., and A. M. Alrubeii. 2024. Effect of elastin hedrolyses on the chemical composition and some oxidation indicators in cold-stored ground beef. *Iraqi Journal of Agricultural Sciences*, 55(2):885-893. <https://doi.org/10.36103/wfj0ra89>
2. Abdullah E. N. 2024. Estimation of contamination with some metallic elements in canned processed meat products available in local markets, *Iraqi Journal of Agricultural Sciences*, 55(4):1502-1507. <https://doi.org/10.36103/3xrbdw07>
3. Al-Khshali, M.S., and N.Y. Karim. 2024. Evaluation of sensory characteristics of some atlantic salmon products imported to Iraq. *Iraqi Journal of Agricultural Sciences*, 55(2), 659-664. <https://doi.org/10.36103/0n7wcs58>
4. Abu-Zahra, N. I. S., A. A. Atia, M. M. Elseify, and Sh. Solima, 2024. Biological and histological changes and DNA damage in *Oreochromis niloticus* exposed to oxytetracycline: a potential amelioratory role of ascorbic acid. *Aquaculture International* 32:3889–3916 . DOI:10.1007/s10499-023-01356-5
5. Ad'hiah, A. H., K. W. Al-Samarraei, and R. M. Al-Ezzy, 2011. Assessing the anti-mutagenic potentials of sage salvia officinal's L. leaf aqueous extract in cultured blood cells of acute lymphocytic leukaemia patients using the micronucleus formation assay. *Journal of Biotechnology Research Center*, 5(2), 25-30. DOI: <https://doi.org/10.24126/jobrc.2011.5.2.158>

6. Al-ghanimi, G. M. M., and A. M. Alrubeii. 2020. Studying the effect of adding different concentrations of astaxanthin and allyl isothiocyanate and their synergistic action in lipid oxidation and some quality characteristics for minced veal meat at cold storage. *Diyala Agricultural Sciences Journal*, 12(Special Issue), 664–674.
<https://doi.org/10.52951/dasj.20121056>
7. Ahmed S.A,D. H. A. Mohammed, S. G. Jassim ,A. F. Hashim and M. J. Laibi. 2017. Detection of antibiotic residues in food animal source and feed. *Iraq J journal of Agricultural Research*, 22, (3):133-139.
8. Al-Kindi. S., I. I. ALBalushi, A. Y. A. Elshaar, A. Al-Kharusi, R.Al-Maimani and A. A. Alka 2023. Determination of Tetracyclines residues in meat available in Oman. *Research Journal of Pharmacy and Technology* .16(5):2182-6
doi: [10.52711/0974-360X.2023.00358](https://doi.org/10.52711/0974-360X.2023.00358)
- 9-Al-Mashhadany. D. A. 2020. Monitoring of antibiotic residues among sheep meats at Erbil city and thermal processing effect on their remnants. *Iraqi Journal of Veterinary Sciences*, 34, (2), 217-222.
DOI: [10.33899/ijvs.2019.125814.1161](https://doi.org/10.33899/ijvs.2019.125814.1161)
9. Al-salmany, A. S. M., and A. M. S. AL-Rubeii. 2020. Effect of cinnamon and turmeric nanoparticles extract in quality characteristics of fresh ground beef during cold storage. *Annals of Tropical Medicine and Public Health*, 23(2): 200–213.
10. Al-Sudany, A. M. 2005. Inhibitory effects of black seed oil and honey on the genotoxicity of aflatoxin in mice. M.Sc Thesis ,College of Science, Al-Nahrain University, Iraq
11. Arsène, M. M. J., A. K. L. Davares, P. I. Viktorovna, S.L. Andreevna, S. Sarra, I. Khelifi and D.M. Sergueïevna, 2022. The public health issue of antibiotic residues in food and feed: Causes, consequences, and potential solutions. *Veterinary World*, 15(3), 662. doi:
<https://doi.org/10.14202/vetworld.2022.662-671>
12. Bacanl, M.G. 2024. The two faces of antibiotics: an overview of the effects of antibiotic residues in foodstuffs. *Arch Toxicol* 98, 1717–1725.
13. Bafana, A., K. Krishnamurthi, S. Sivanesan and P.K. Naoghare, 2018. Mutagenicity and genotoxicity testing in environmental pollution control. *Mutagenicity: Assays and Applications* (pp. 113-132). Academic Press.
DOI: [10.1016/B978-0-12-809252-1.00006-7](https://doi.org/10.1016/B978-0-12-809252-1.00006-7)
14. Bedale, W. A., A.L. Milkowski, C.J. Czuprynski and M.P. Richards, 2023. Mechanistic Development of Cancers Associated with Processed Meat Products: A Review. *Meat and Muscle Biology*, 7(1): 15762, 1–63 (2023).
doi:10.22175/mmb.15762
15. Breijyeh Z., B. Jubeh and R. Karaman, 2020. Resistance of Gram-Negative Bacteria to Current Antibacterial Agents and Approaches to Resolve It. *Molecules*. 25:1340. doi: [10.3390/molecules25061340](https://doi.org/10.3390/molecules25061340).
16. Bobate, S., S. Mahalle, N.A. Dafale, and A. Bajaj, 2023. Emergence of environmental antibiotic resistance: Mechanism, monitoring and management. *Environmental Advances*, 13;100409.
<https://doi.org/10.1016/j.envadv.2023.100409>
17. Collins, A., P. Møller, G. Gajski, S. Vodenková, A. Abdulwahed, D. Anderson and A. Azqueta, 2023. Measuring DNA modifications with the comet assay: a compendium of protocols. *Nature Protocols*, 18(3), 929-989. doi:10.1038/s41596-022-00754-y. Epub 2023 Jan 27.
18. Cvetković, V. J., T. D. Miladinov and S. Stojanović, 2018. Genotoxicity and mutagenicity testing of biomaterials. *Biomaterials in clinical practice: Advances in Clinical Research and Medical Devices*, 501-527. Springer International Publishing.
DOI: [10.1007/978-3-319-68025-5_18](https://doi.org/10.1007/978-3-319-68025-5_18)
19. Er, B., F. K. Onurdağ, B. Demirhan, S. O. Özgacar, A.B. Öktem and U. Abbasoğlu, 2013. Screening of quinolone antibiotic residues in chicken meat and beef sold in the markets of Ankara, Turkey. *Poultry Science*, 92(8), 2212-2215.
doi: [10.3382/ps.2013-03072](https://doi.org/10.3382/ps.2013-03072).
20. Fahim, H. M. 2019. Evaluate antibiotic residues in beef and effect of cooking and freezing on it. *Benha Veterinary Medical Journal*, 36(2), 109-116.

DOI: [10.21608/bvmj.2019.13927.1029](https://doi.org/10.21608/bvmj.2019.13927.1029)

21. Fenech M. 2000. The invitromicronucleustechnique. *Mutat Res.*20;455(1-2):81-95.

doi:10.1016/s0027-5107(00)00065-8.

PMID: 11113469.

22. Fei, Z., S. Song, X. Yang, D. Jiang, J. Gao and D. Yang, 2022. Occurrence and risk assessment of fluoroquinolone residues in chicken and pork in China. *Antibiotics*, 11(10), 1292.

<https://doi.org/10.3390/antibiotics11101292>

23. Galdíková, M., V. Schwarzbacherová, and M. Drážovská, 2020. Genotoxicity testing of bovine lymphocytes exposed to epoxiconazole using alkaline and neutral comet assay. *Caryologia*, 73(4), 99-109.

DOI:

<https://doi.org/10.13128/caryologia-984>

24. Geffré A, D Concordet, J.P Braun and C. Trumel, 2011. Reference Value Advisor: a new freeware set of macroinstructions to calculate reference intervals with Microsoft Excel. *Vet Clin Pathol.* Mar;40(1):107-12. doi: 10.1111/j.1939-165X.2011.00287.x. Epub 2011 Feb 7. PMID: 21366659.

25. Ghimpețeanu, O. M., E. N. Pogurschi, D. C. Popa, N. Dragomir, T. Drăgotoiu, O.D. Mihailescu and C.D. Petcu, 2022. Antibiotic use in livestock and residues in food—A public health threat: A review. *Foods*, 11(10), 1430.

<https://doi.org/10.3390/foods11101430>

26. Hou, L., Y. Fu, C. Zhao, L. Fan, H. Hu and S. Yin, 2024. The research progress on the impact of antibiotics on the male reproductive system. *Environment International*, 108670.

<https://doi.org/10.1016/j.envint.2024.108670>

27. Jabbar, A. and S. Ur-Rehman. 2013. Microbiological evaluation of antibiotic residues in meat, milk and eggs. *Journal of Microbiology, Biotechnology and food Sciences*, 2(5), 2349-2354.

28. Jain, A. K., D. Singh, K. Dubey, R. Maurya and A. K. Pandey, 2018. Chapter Four: Chromosomal aberrations. *Mutagenicity: Assays and Applications* (pp. 69-92). Academic Press.

<https://doi.org/10.1016/B978-0-12-809252-1.00004-3>

29. Jones, G. M., and E. H. Seymour, 1988.

Cow side antibiotic residue testing. *Journal of dairy science*, 71(6), 1691-1699. [https://doi.org/10.3168/jds.S0022-0302\(88\)79734-9](https://doi.org/10.3168/jds.S0022-0302(88)79734-9)

30. Jutzi, S. 2004. Good practices for the meat industry (No. 2). Food & Agriculture Organization of the United Nations. Rome..

<https://openknowledge.fao.org/handle/20.500.14283/y5454e>

31. Kyuchukova, R. 2020. Antibiotic residues and human health hazard-review. *Bulgarian Journal of Agricultural Science*, 26 (No 3) 2020, 664–668. <https://www.researchgate.net/publication/342766423>

32. Ma, F., S Xu, Z. Tang and Z, Li, L, Zhang. 2021. Use of antimicrobials in food animals and impact of transmission of antimicrobial resistance on humans. *Biosafety and Health*, 3(1): 32-38.

<https://doi.org/10.1016/j.bsheal.2020.09.004>

33. Maddim S., T. Goud and P. Srivastava, 2017. Cytochrome p450 Enzymes, Drug Transporter and their role in Pharmacokinetic Drug- Drug interactions of Xenobiotics: A comprehensive review, *J. Med Chem Res* 3(1):1-11.

DOI: <http://dx.doi.org/10.17352/ojc.000006>

34. Med Calc Statistical Software version 16.4.3 (MedCalc Software bvba, Ostend, Belgium; <https://www.medcalc.org>; 2016)"

35. Monger, X. C., A. A. Gilbert, L. Saucier and A. T. Vincent, 2021. Antibiotic resistance: From pig to meat. *Antibiotics*, 10(10), 1209.

<https://doi.org/10.3390/antibiotics10101209>

36- Majd A. A, A. M. S. Alrubeii, and L. T. Al-Hadedee. 2025. Using of electrospun chromium oxide nanofiber to increase the shelf life of frozen beef burger. *Iraqi Journal of Agricultural Sciences*, 56(Special), 102-110. <https://doi.org/10.36103/2x991f27>

37. Murray, C. F., Fick, L. J., Pajor, E. A., Barkema, H. W., Jelinski, M. D., and Windeyer, M. C. 2016.

Calf management practices and associations with herd-level morbidity and mortality on beef cow-calf operations. *Animal*, 10(3), 468-477. DOI: [10.1017/S1751731115002062](https://doi.org/10.1017/S1751731115002062)

38. Najim N. H., K. F. Khazal and J.H. Ali, 1988. Antibiotics residues in milk of Baghdad area. *The Iraqi Journal of*

- Veterinary Medicine, 12(1),32-38.
<https://doi.org/10.30539/ijvm.v12i1.1682>
39. Najim. N. H and S. A. Al-Kurashi. 2017. Detection of antibiotic residues in locally raw milk by using high performance liquid chromatography at different seasons and the effect of heat treatment on their concentration. The Iraqi Journal of Veterinary Medicine, 41(2):131-136. DOI: <https://doi.org/10.30539/iraqijvm.v41i2.62>
40. Ngangom., B. L, Tamunjoh, S. S. A and Boyom, F. F. 2019. Antibiotic residues in food animals: Public health concern. Acta Ecol Sin 39(5):411–415.
<https://doi.org/10.1016/j.chnaes.2018.10.004>
41. Orozco-Hernandez, J.M., L.M. Olivan, G. Heredia-García, M. Luja-Mondragon, H. Islas- Flores, H., N. San Juan-Reyes and M. Galar-Martínez, *et al.* 2019. Genotoxic and cytotoxic alterations induced by environmentally-relevant concentrations of amoxicillin in blood cells of
42. Patel, S.J., M. Wellington, and R.M. Shah and M.J. Ferreira, 2020. Antibiotic stewardship in food-producing animals: challenges, progress, and opportunities. Clinical Therapeutics, 42(9), 1649-1658. DOI: [10.1016/j.clinthera.2020.07.004](https://doi.org/10.1016/j.clinthera.2020.07.004)
43. Rahman M. S., M. M. Hassan, and S. Chowdhury, 2021.. Determination of antibiotic residues in milk and assessment of human health risk in Bangladesh. Heliyon. 8;7(8):e07739.
 doi: [10.1016/j.heliyon.2021.e07739](https://doi.org/10.1016/j.heliyon.2021.e07739). PMID: 34430734; PMCID: PMC8365374.
44. Ramatla, T., L., Ngoma and M. Adetunji, and M. Mwanza, 2017. Evaluation of antibiotic residues in raw meat using different analytical methods. Antibiotics, 6(4), 34.
<https://doi.org/10.3390/antibiotics6040034>
45. Ramadhani, D., S. Purnami, D. Tetriana, I. Sugoro, V.A. Suvifan, N. Rahadjeng and M. Syaifudin, 2023. Chromosome aberrations, micronucleus frequency, and catalase concentration in a population chronically exposed to high levels of radon. International Journal of Radiation Biology, 99(8), 1188-1203.
 DOI: [10.1080/09553002.2022.2110314](https://doi.org/10.1080/09553002.2022.2110314)
46. SAS. 2010. SAS/STAT Users Guide for Personal Computer. Release 9.13. SAS Institute, Inc., Cary, N.C., USA.
47. Sarkar, S., M. J. Souza, T. Martin-Jimenez, M. A. Abouelkhair, S. A. Kania and C. C. Okafor, 2023. Tetracycline, Sulfonamide, and Erythromycin Residues in Beef, Eggs, and Honey Sold as “Antibiotic-Free” Products in East Tennessee (USA) Farmers’ Markets. Vet. Sci. 10,243.
<https://doi.org/10.3390/vetsci10040243>
48. Seo, J., F. Kloprogge, A. M. Smith, K. Karu and K., L. Ciric, 2024. Antibiotic Residues in UK Foods: Exploring the Exposure Pathways and Associated Health Risks. Toxics, 12(3), 174.
<https://doi.org/10.3390/toxics12030174>
49. Shubber E. K. 1987. Sister-chromatid exchanges in lymphocytes from patients with Schistosoma hematobium. Mutat Res. 180(1):93-9. doi: [10.1016/0027-5107\(87\)90071-6](https://doi.org/10.1016/0027-5107(87)90071-6). PMID: 3114626.
50. Silva-Guedes, J., A. Martinez-Laorden and E. Gómiz-Fandos, 2022. Effect of the presence of antibiotic residues on the microbiological quality and antimicrobial resistance in fresh goat meat. Foods, 11(19):3030;
<https://doi.org/10.3390/foods11193030>
51. Treiber, F. M. and H. Beranek-Knauer, 2021. Antimicrobial residues in food from animal origin—A review of the literature focusing on products collected in stores and markets worldwide. Antibiotics, 10(5), 534.
<https://doi.org/10.3390/antibiotics10050534>
52. Wali, M. K., and A.H. Deri, 2022. Effect of thermal processing on antibacterial drug residue of tetracycline and sulfonamide in fresh beef meat and Iraqi processed meat. Intl J Health Sci. 6(S2)
<https://doi.org/10.53730/ijhs.v6nS2.6701>
53. Zhang , Y., J. Lu, Y. Yan, J. Liu and M. Wang, 2021. Antibiotic residues in cattle and sheep meat and human exposure assessment in southern Xinjiang, China. Food Sci Nutr, 9(11):6152-6161.
 doi: <https://doi.org/10.1002/fsn3.2568>